UNIVERSITY OF PENNSYLVANIA

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WILLIAM DUDLEY GRAY

A DISSERTATION

IN BOTANY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

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THE EFFECT OF LIGHT ON THE FRUITING OF MYXOMYCETES [1]

William D. Gray

Although the myxomycetes are unique in that they unite in themselves a number of characteristics of both animals and plants, they have been investigated by relatively few biologists. As a result of this neglect there are many gaps in our knowledge of their simpler physiological processes, and a search through the literature reveals the fact that little is known of them except perhaps the taxonomy within the group. This situation cannot be attributed to a lack of interest or inclination on the part of biologists, although it might be explained to some extent by the rather uncertain position of the myxomycetes. One reason for so little being known is probably the fact that only a few species have been cultured. Our present knowledge of the biology of this group has been obtained from the few forms which can be easily cultured in the laboratory and from observations made on species collected in their vegetative phase. The limitations of this latter procedure are obvious: No matter how assiduous a worker may be, there are many species which he may never see in the vegetative state; in fact, the plasmodia of only about one-third of the described species have ever been seen and recorded. The problem that immediately confronts us then is that of culturing species of myxomycetes which have never been grown in the laboratory.

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Certain species have been cultivated successfully, but these form a very small percentage of the total. *Physarum polycephalum* Schw. can be most satisfactorily cultured at present. Howard (1931) first successfully cultured this species in the laboratory, and further improvements by Camp (1936) made the cultivation of *P. polycephalum* much simpler than that of most fungi. Skupienski (1920, 1928) has cultured both *Didymium difforme* (Pers.) Duby and *D. nigripes* (Link.) Fr. on potato and carrot decoction agar, and Lister (1888) has described the cultivation of *Badhamia utricularis* (Bull.) Berk. on the sporophores of various agarics. The limitation of Lister's method is obviously the fact that such sporophores are not available at all times of the year. Klebs (1900) cultured *D. difforme* and *D. effusum* (synonym of *D. squamulosum* (Alb. & Schw.) Fr.), and Cayley (1929) cultured *D. nigripes* and *D. xanthopus* (Ditm.) Fr., in addition to the two species of *Didymium* used by Klebs, on Knop agar. A few other forms have also been cultured, but, considering the fact that there are about four hundred species of myxomycetes, it is clear that comparatively few have been grown in the laboratory.

Many attempts of the writer to cultivate various species (largely of the order Physarales) from living plasmodia, collected in their natural habitats, have generally failed because of the rapid formation of fruiting bodies by the plasmodia when brought into the laboratory, despite efforts to simulate conditions

under which they were growing in nature. Such results lead to the suggestion, already advanced by Cayley (1929) and Seifriz and Russell (1936), that there is a definite rhythmical fruiting exhibited by myxomycetes. Cayley found that *Didymium difforme* possesses a much shorter vegetative phase than either *D. nigripes* or *D. squamulosum* but stated that the factors causing plasmodia to form fruiting bodies are "still a complete mystery" (p. 234). If such rhythmical fruiting does exist, it is highly possible that the species observed by the writer may possess rhythms of relatively short duration in so far as the vegetative phase is concerned. Unless this rhythm is so innate as to be unalterable, it should be possible, by controlling various environmental factors, to vary the rhythm one way or the other so that plasmodia would vegetate for a longer or shorter period than normal. Thus it is entirely possible that a larger number of species might be brought into and maintained in cultivation if methods of prolonging the vegetative phase might be found. Of course, the problem of nutrition must also be considered, but even if the nutritional requirements of a species be known, it would be an extremely arduous task to maintain it in culture if it remained in the vegetative state for only a short period. Undoubtedly, a plasmodium about to form fruiting bodies differs fundamentally from one which is beginning its vegetative existence, even though the exact nature of the difference is not known. If the change from vegetative to fruiting phase is due to chemical factors, physical factors, or a combination of both, it should be possible to hasten or delay it. As mentioned above, the factor of nutritional requirements is an important one, and no attempt is made to minimize it; however, this can undoubtedly be ascertained by experimentation with various species.

The present series of experiments are concerned with the influence of light upon the length of time required to develop fruiting bodies in various species of myxomycetes. There is a possibility that these findings may have a bearing upon the culture of other species as well as contribute to our knowledge of the physiology of the group. It is quite probable that temperature, moisture, texture of substratum, nutrient materials, and degree of acidity or alkalinity of the medium are also important, but in the present work the influence of light alone is described.

Almost no investigations have been made of the effects of light upon myxomycete plasmodia. The most extensive work in this field was that Baranetzki (1876), who, however, limited himself almost entirely to studies of the heliotropic responses of plasmodia of *Fuligo septica* (L.) Weber and the interrelationships of heliotropic and geotropic responses. The remaining references in the literature are merely scattered observations made by various workers in the course of other studies; these observations will be referred to in the discussions of the experiments conducted by the writer.

Materials and methods.—With the exception of plasmodia of *Physarum polycephalum* Schw., which were derived from sclerotia, plasmodia of all species cultured in the laboratory were obtained from spore cultures. Since there is not enough surface moisture on agar slants to insure good spore germination, cultures were made by adding 1 cc. of sterile distilled water to each slant; spores were then sown directly in the water. Such cultures were placed in the dark, as various workers have shown that light destroys swarm cells. When plasmodia developed, they were maintained in culture by means of plasmodial transplants to fresh nutrient media. Three culture methods were used: (1) the moist chamber method developed by Camp (fig. 7), (2) Petri plate culture (fig. 13), and (3) beaker culture (fig. 9). With *P. polycephalum*, all three methods were employed, whereas only Petri dish cultures were made for *P. tenerum* Rex, *P. compressum* Alb. & Schw., *Didymium xanthopus* (Ditm.), Fr., *Hemitrichia vesparium* (Batsch) Macbr., *Fuligo septica* (L.) Weber, and *Leocarpus fragilis* (Dicks.) Rost.

In moist chamber cultures of *P. polycephalum*, the plasmodia were fed every twenty-four hours by sprinkling ground-up rolled oats directly on them; in both beaker and Petri dish cultures of this same species, three per cent rolled oat agar was used. *P. tenerum*, *P. compressum*, *F. septica*, and *H. vesparium* were grown on two and five-tenths per cent corn decoction agar, adjusted to pH 5.4–5.6; *D. xanthopus* was grown on five per cent carrot decoction agar; *L. fragilis* was grown on both corn decoction agar and three per cent hay infusion, one per cent dextrose agar.

In experiments involving light intensity, the cultures were placed under light bulbs of various wattage, the bulbs being suspended three feet above the open top boxes in which the cultures were placed. All experiments involving such artificial light were conducted in a room in which the temperature varied from 23° to 26°C. Experiments involving direct sunlight were conducted in a greenhouse where the temperature remained at 21°C. in the daytime, dropping to as low as 15°C. at night.

Species herein considered are divided into three groups, based on plasmodial color: yellow pigmented, non-pigmented, and variable plasmodial types, which ranged from white to cream-colored and various light shades of yellow.

Yellow-pigmented plasmodial types.—This group includes the species whose plasmodia are normally yellow at all times, except for the period immediately previous to the formation of fruiting bodies. In this group are included *Physarum polycephalum* Schw., *P. tenerum* Rex., *Fuligo septica* (L.) Weber, and *Leocarpus fragilis* (Dicks.) Rost.

Physarum polycephalum Schw.—It was possible to conduct many more experiments with this species than with any of the others, because of the ease with which it can be cultured, the rapidity of its growth, and the subsequent formation of relatively large

amounts of protoplasm. As has been previously mentioned, all three types of culture methods were utilized, but the majority of experiments was conducted with moist chamber cultures which were fed equal amounts (0.5 gm.) of pulverized rolled oats every twenty-four hours. It was soon discovered that the plasmodia could not be kept in direct sunlight, although whether the destruction was due to the intensity or quality of the light is not yet known; possibly both factors were concerned.

Preliminary experiments revealed that, except in a few cases, plasmodial cultures of the beaker or Petri dish type would not form fruiting bodies in the complete absence of light if they were inoculated from stock cultures that had been maintained in the dark. This was also found to be true for moist chamber cultures that were fed every twenty-four hours. Several hundred cultures of this type were set up in the dark at various times and only three or four fruited, while check cultures grown in artificial or diffuse natural light nearly always formed sporangia. Those few cultures that did fruit in the dark passed a considerable time (15 days or over) in the vegetative stage. In cultures inoculated from stock plasmodia maintained in strong natural light, a delayed effect was frequently shown, for cultures of this type often fruited in the complete absence of light. Sixty cultures were inoculated from stock plasmodia which had been kept in the light; of these, seventeen (28 per cent) produced sporangia in the dark. In order to overcome any delayed effect, all subsequent experiments were conducted with cultures inoculated from stock plasmodia maintained in the dark. For the sake of uniformity, five-day old stock cultures were arbitrarily chosen as sources of inocula.

Plasmodia grown in light were always a paler yellow than those kept in the dark. Hofmeister (1867, p. 21) stated that plasmodia of *Fuligo septica* (L.) Weber which were kept in the dark were paler than those grown in the light; however, Baranetzki (1876), working with the same species, stated that the reverse was true. The observations of the writer on yellow-pigmented plasmodia tend to confirm the findings of Baranetzki rather than those of Hofmeister. It was also noted in *P. polycephalum* that plasmodia grown in the dark showed a tendency to spread, whereas the protoplasm of plasmodia grown in light showed a decided tendency to form clumps and large, swollen plasmodial "veins." These two types of plasmodial configurations are shown in figures 4 and 5.

The finding that light was nearly always essential for the fruiting of this species, under the conditions outlined, naturally suggested that the intensity of light might condition the time of fructification, and it was subsequently found that a definite correlation exists between light intensity and duration of vegetative phase. Over a period of two years moist chamber cultures were grown in open top boxes under 60, 100, 150, 200, and 400 (two 200's) watt bulbs, suspended three feet above the cultures, and it was found that under each type of light (with the exception of the most intense) a rather definite fruiting rhythm was exhibited. The following list summarizes these data:

Cultures under 60 watt light fruited in 12 days
" " 100 " " " " 11 "
" " 150 " " " " 10 "
" " 200 " " " " 9 "
" " 400 " " " " 6–9 "

There were some variations from the above figures, but, in the great majority of cases, the fruiting occurred with such regularity that the day of sporangium formation of a given plasmodial culture could be correctly predicted on the day it was started. The analyses of lights from the various types of bulbs used in the experiments show that variations in the length of time spent in the vegetative phase were due to the factor of intensity rather than to differences in quality of the various lights. Analyses of the types of bulbs used are shown in table 1.[2]

TABLE 1. *Analyses of electric light bulbs used in light intensity studies.*

Lamp size (watts)	Approximate lumens	Percentage of total light			
		Violet and blue	Green	Yellow	Orange and red
60	760	2.25	40.25	27.5	30.0
100	1530	2.3	40.7	27.4	29.6
150	2535	1.9	41.0	27.0	30.1
200	3400	2.5	41.5	27.2	28.8

In discussing various features of the plasmodial behavior of *Didymium difforme* (Pers.) Duby, Skupienski (1928, p. 329) made the statement: ". . . . if one submits a well-expanded plasmodium to the action of light rays, coming from a well-defined source, for two minutes only, and if one repeats this short illumination every two hours, the fruiting of the plasmodium in question is surely accelerated." This suggested the possibility that fruiting might be induced more rapidly by alternate periods of light and darkness rather than by continuous exposure. Moist chamber cultures were exposed to light from a 100 watt bulb for eight out of each twenty-four hours and were found to require one to two days longer for fruiting than those cultures continuously exposed to the same light. Cultures which were exposed to intermittent illumination from a 60-watt bulb (obtained by means of a flashing device that gave periods of 2.5 seconds of light alternating with periods of 0.5 second of darkness), remained in the vegetative state two days longer than cultures exposed to continuous illumination from the same type of bulb. It would seem from these results that alternate periods of light and darkness enable plasmodia to fruit with a much smaller total amount of light than required by plas-

[2] From the manufacturer's published tables.

modia when they are continuously exposed. The cultures exposed to light from a 100-watt bulb for eight hours per day required only one or two days longer for fruiting than cultures continuously exposed, whereas, if a direct mathematical relationship existed, theoretically they would require thirty-three days, since they were exposed to light only one-third of the time. What actually occurred was that cultures continuously exposed required a total of 264 hours exposure to light for fruiting, while cultures exposed eight hours per day required only 96 to 104 hours of exposure, although they did spend a longer time in the vegetative phase than cultures continuously exposed.

TABLE 2. *Light transmission analyses of colored plates.*

	Wave length (millimicrons)						
Plate used	400	450	500	550	600	650	700
Blue	68.00	45.45	6.46	2.56	0.57	0.52	24.46
Red	6.00	0.56	3.29	3.84	16.09	33.68	43.61
Yellow ..	4.00	2.27	10.52	21.79	33.33	35.78	39.36

Experiments relative to the effect of lights of various wave lengths (of the visible spectrum) were first conducted with moist chamber cultures which were continuously exposed to lights transmitted through colored glass plates. One series was conducted, and then standardized gelatin filters were substituted for the glass plates. In the original experiment three colored plates were used: red, blue, and yellow. These plates were analyzed by means of a Razek-Mulder Color Analyzer; results of the light transmission analyses are summarized in table 2. From this table it may be seen that the blue plate passed a comparatively high percentage of light in the violet and blue bands of the spectrum, very little in the green and yellow, practically none in the orange, but

TABLE 3. *Summary of results of one experiment using light transmitted through variously colored plates.*

Culture number	Color of plate used	Hours per day exposed	Length of vegetative phase
1	Blue	24	15 days
2	"	8	14 "
3	Red	24	Died
4	"	8	16 days
5	Yellow	24	Died
6	"	8	14 days

passed considerable light in the red and near infra red. The red plate passed very little in the violet and blue bands, from 3.9 to 16 per cent in the green and yellow, increasing in the orange and red. The light transmission of the yellow plate was roughly similar to that of the red plate; however, it transmitted a greater percentage of light in the green, yellow, and orange bands.

Six cultures were used in the preliminary experiment, the results of which are given in table 3. This experiment showed that the shorter wave lengths of the visible spectrum stimulate fruiting, while the longer wave lengths either retard or prevent fruiting. In later experiments in which Wratten Filters were used, only one, whose transmission analysis is shown in figure 1, induced fruiting. This would tend to support the view that the shorter wave lengths are necessary for fruiting. On the other hand, in the longer wave lengths of the visible spectrum or in infra-red rays, the plasmodia never fruited under conditions of continuous exposure.

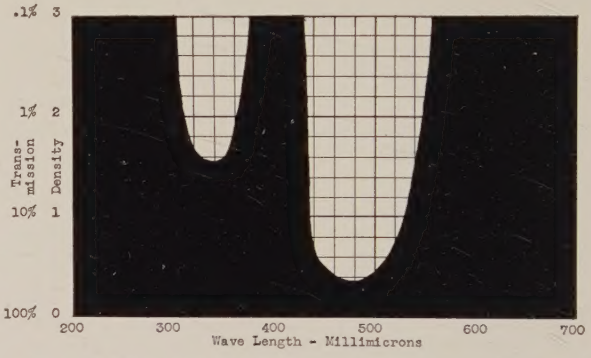


Fig. 1. Light transmission analysis of gelatin filter under which fed cultures of *P. polycephalum* formed fruiting bodies.

A number of investigators have maintained that the fruiting of myxomycetes is due solely to the depletion of nutrition. Camp (1937) has shown that plasmodia of *P. polycephalum* may be induced to fruit by starvation. The present study indicates that cultures which are not fed will spend less time in the vegetative phase than those which are fed, but this will scarcely explain why plasmodia will

TABLE 4. *The effect of light intensity on fruiting in unfed cultures of Physarum polycephalum.*

Lamp (watts)	Number of cultures	Average length of time required for fruiting
60 W.	8	87 hours
100 W.	6	81 "
150 W.	21	78 "
400 W.	21	68 "

often fruit when they are supplied with abundant food materials. In order to determine whether or not light would have any effect on plasmodia to which no nutrient was supplied, a number of cultures were set up. As in previous experiments, five-day old stock cultures, which had been fed twenty-four hours previously, were used as sources of inocula. With few exceptions the light reactions were the same as those

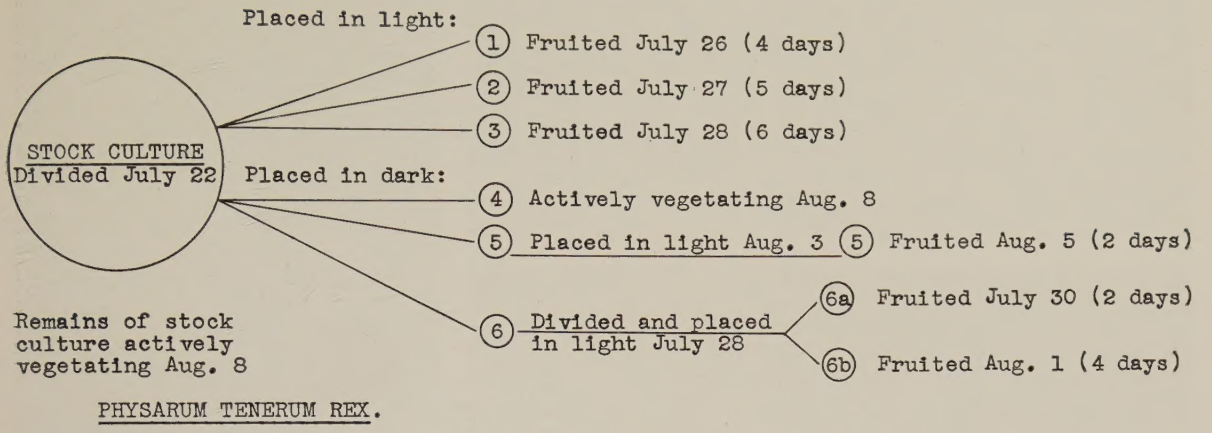

Placed in light:
1 Fruited July 26 (4 days)
2 Fruited July 27 (5 days)
3 Fruited July 28 (6 days)
STOCK CULTURE
Divided July 22
Placed in dark:
4 Actively vegetating Aug. 8
5 Placed in light Aug. 3
5 Fruited Aug. 5 (2 days)
6a Fruited July 30 (2 days)
Remains of stock culture actively vegetating Aug. 8
6 Divided and placed in light July 28
6b Fruited Aug. 1 (4 days)
PHYSARUM TENERUM REX.

Fig. 2. Diagrammatic representation of the effect of diffuse natural light upon the fruiting of *P. tenerum*.

shown by fed cultures. A higher percentage of unfed cultures (inoculated from stock plasmodia maintained in the dark) formed sporangia in the complete absence of light than did fed cultures of the same type. Results of light intensity studies conducted with cultures of this type are summarized in table 4.

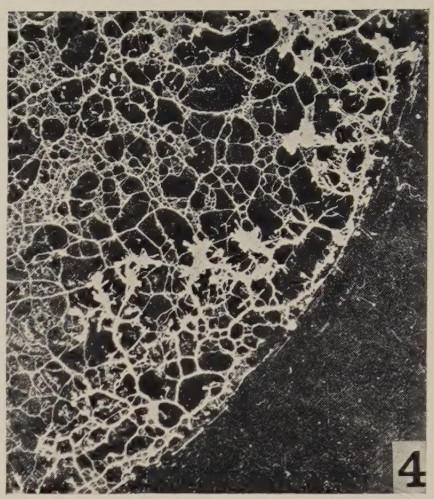
Experiments concerned with exposing unfed cultures to various wave lengths of light did not produce results which were in agreement with the results obtained when fed cultures were used. Sporadic fruiting occurred in such cultures, regardless of the light used, so the inference is that the influence of starvation is much stronger than any possible inhibitory effect of light in the longer wave bands.

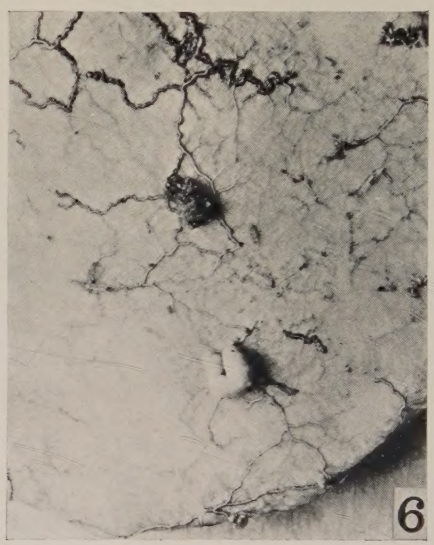
Physarum tenerum Rex.—This species has the same type of bright yellow plasmodium as *P. polycephalum*, but as yet has not been induced to form large masses of protoplasm. Like *P. polycephalum*, plasmodia of *P. tenerum* grown in the light were a paler shade of yellow than those grown in the dark. A clumping of plasmodia occurred in the light but to a lesser degree than in *P. polycephalum*.

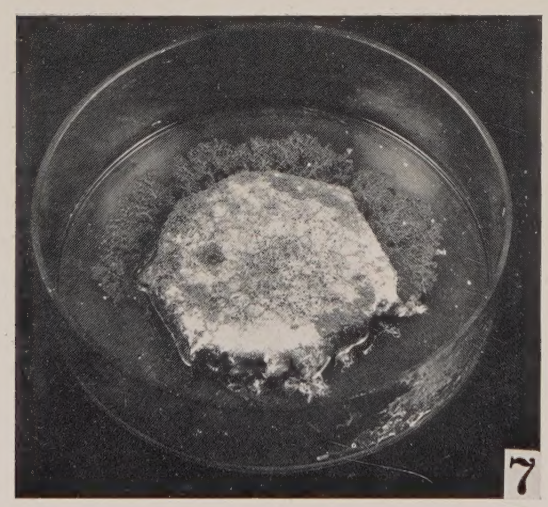
Numerous plasmodial cultures were exposed to direct sunlight in the greenhouse, and after forty-eight hours all had lost their color. When these cultures were placed in the dark, they failed to revive, thus showing that plasmodia of *P. tenerum*, like those of *P. polycephalum*, are killed by direct sunlight. In no case did plasmodial cultures fruit in the complete absence of light; over two hundred cultures were kept in the dark, and all remained in the active vegetative state as long as the nutrient agar remained moist. Many plasmodia remained active for as long as sixty days, and one persisted for seventy-six. Due to the fact that it needs transplanting so infrequently, this species is excellently adapted for laboratory cultivation, although, as mentioned above, it does not produce such large quantities of protoplasm as *P. polycephalum*. Preliminary experiments indicate that it can probably be adapted to moist chamber culture, using pulverized rolled oats as nutrient.

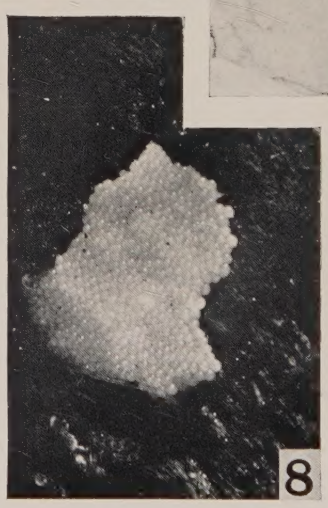
In diffuse natural light in the laboratory, different lengths of time were required for the formation of fruiting bodies by various plasmodial cultures. The results of one such experiment are shown in figure 2. This figure shows that the vegetative phase of *P. tenerum* varied from 2 to 6 days in natural light and well illustrates the necessity for more controlled conditions than are normally found in the laboratory. Seifriz and Russell (1936) apparently encountered similar difficulties, since they found that under laboratory conditions the rhythm of *P. polycephalum* varied from 9 to 23 days — a situation non-existent for this species (*polycephalum*) under conditions of constant light, temperature, and food supply. Under such controlled conditions some variation did occur in cultures of *P. tenerum*, but this variation was not nearly so great as that exhibited by cultures grown in natural light. The average length of the vegetative phase of twenty-four cultures grown under a 100-watt light was eighteen days, while under a 400-watt light the average length of the vegetative phase was thirteen days (15 cultures used). This difference is sufficient to show that the same relation exists between length of vegetative phase and light intensity as was exhibited by *P. polycephalum*. Plasmodia of *P. tenerum* possess a vegetative phase of somewhat longer duration than plasmodia of *P. polycephalum*.

Fuligo septica (L.) Weber.—Spores of this species germinate readily, and small, yellow plasmodia have frequently been obtained from spore sowings on corn decoction agar. These plasmodia, which failed to attain the size of those found in nature, grew very slowly, and none was ever observed to complete its life cycle. Attempts to maintain this species in moist chamber culture for any length of time have also failed; hence, all observations on this species have necessarily been made on plasmodia collected in the field. From these observations alone, it would seem that *F. septica* must have light in order to complete its life cycle, because young plasmodia, which were collected in more or less shaded habitats and kept in darkness, have never been observed to form fruiting bodies.









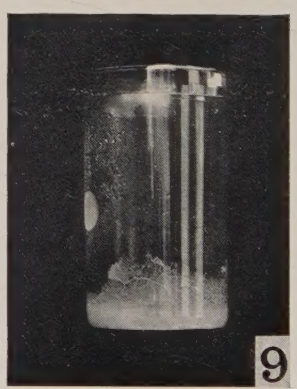
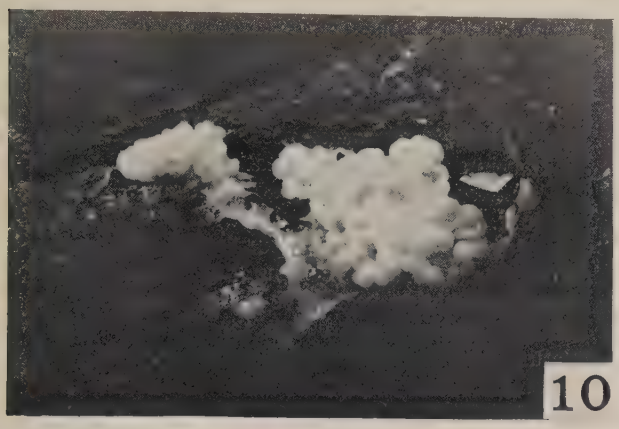


Fig. 3–9.—Fig. 3. Immature sporangia of *H. clavata*. × 6.—Fig. 4. Portion of plasmodium of *P. polycephalum*, grown in darkness. Natural size.—Fig. 5. Portion of plasmodium of *P. polycephalum*, grown in light. Natural size.—Fig. 6. Plasmodium of *F. septica*, showing clumping of protoplasm. × 1½.—Fig. 7. Moist chamber culture of *P. polycephalum*. × ⅓.—Fig. 8. Immature sporangia of *T. persimilis*. × 2.—Fig. 9. Beaker culture of *P. polycephalum*. × ¼.

Plasmodia of *F. septica* show the phenomenon of clumping in a remarkable degree when exposed to light. For this study, pieces of wood, on which active plasmodia were spread, were placed in moist chamber culture, and in a few hours most of the plasmodia had moved to the moist paper on which the pieces of wood were placed. When such plasmodia were exposed to light from a 100-watt bulb, at a distance of eighteen inches, a noticeable congregation of protoplasm into the larger veins of the plasmodial network became apparent at the end of one minute. At various points along these veins, swellings began to appear, and four to five minutes later some of them had spore sowings on either corn decoction agar or hay infusion agar. These plasmodia need transplanting infrequently (every 2 or 3 weeks) and hence are easily maintained. No fruiting has been obtained as yet, and the suggestion is made that neither of the two media used is adapted for its cultivation. Like the yellow-pigmented types previously discussed, the plasmodia of *L. fragilis* clump and are bleached when exposed to light and are killed when exposed to direct sunlight.

Non-pigmented plasmodial types.—In this group are placed those species in which the plasmodia are typically watery- or pearly-white. All species in the



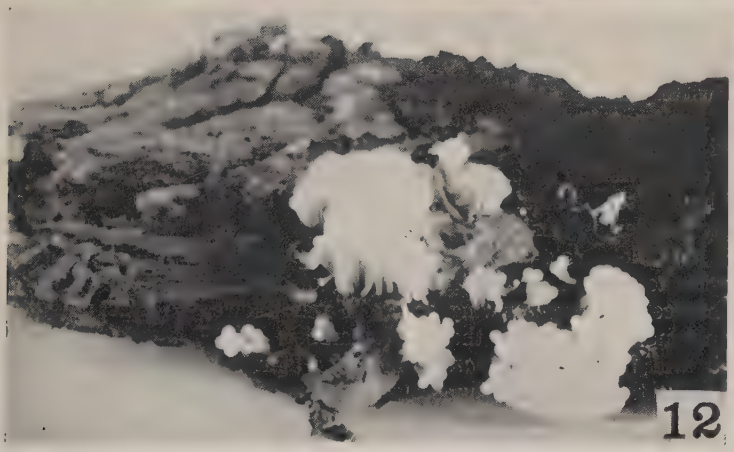


Fig. 10–13.—Fig. 10. Plasmodium of *S. fusca*. × 3.—Fig. 11. Same as fig. 10 — 6½ hours later. × 3.—Fig. 12. Same as fig. 10 — 4½ hours later. × 3.—Fig. 13. Petri dish culture of *D. xanthopus* on carrot agar. Natural size.

grown as large as 1 cm. in diameter (fig. 6). Baranetzki (1876) mentioned the clumping of plasmodia of *F. septica* exposed to light but failed to mention the production of large plasmodial swellings such as those just described. Plasmodial cultures of *F. septica* on corn decoction agar were killed within twenty-four hours when exposed to direct sunlight.

Leocarpus fragilis (Dicks.) Rost. — In so far as a source of small plasmodia is concerned, this species is an excellent yellow-pigmented type for laboratory cultivation. Spores germinate readily, and plasmodia can generally be obtained in ten days or less from group herein discussed change color before the fruiting bodies mature, but the change occurs after sporangial delimitation has begun.

Physarum compressum Alb. & Schw.—The pearly-white plasmodium of this species was obtained easily from spore sowings on corn decoction agar. Plasmodia formed typical sporangia in the absence of light about six days after they were large enough to be visible without the aid of a lens. Attempts to maintain the species in culture by means of plasmodial transplants were, on the whole, unsuccessful. Disturbing the plasmodia markedly inhibits growth

and fruiting, and cultures made by means of plasmodial transplants nearly always died within twenty-four hours, although in a few cases the life cycle was completed. Either the plasmodium of *P. compressum* is exceptionally delicate or else corn decoction agar is not the most suitable medium for its cultivation. However, attempts to grow this species on various other types of media yielded no better results.

Physarum nutans Pers.—Only one observation was made on the light relationships of this species. A small, white, net-like plasmodium, found under the bark of a decaying log of *Prunus serotina*, was transported to the laboratory, kept in complete darkness, and, after three days, formed typical sporangia of *P. nutans*. The plasmodium received no light except for a few moments when it was first discovered, so it is safe to infer that light is not prerequisite for the completion of its life cycle. Since the age of the plasmodium was unknown, no definite length of vegetative phase may be set.

Stemonitis spp.—The plasmodia of approximately half of the described species in the genus *Stemonitis* have not yet been observed, but in the majority of known cases they are white. This is true for both *Stemonitis axifera* (Bull.) Macbr. and *S. fusca* Roth (fig. 10, 11, 12), the plasmodial behavior of which has been described previously by the writer (Gray, 1936, 1937). All conclusions concerning these species are based on observations made on plasmodia collected in their natural habitats, as there are no records of either species having been artificially cultured, and to date no methods for their cultivation have been devised. From observations made by the writer, it is evident that light is not necessary for fruiting, since plasmodia which arose on decayed wood in moist chambers were able to complete their life cycles in the absence of light. Several observations have also been made on plasmodia which were collected under the bark of decaying logs and transported in darkness to the laboratory; fruiting of such plasmodia subsequently occurred in the complete absence of light. As in other species, in which the plasmodia inhabit the interstices of decaying wood, it will be difficult to establish a definite length of time for the vegetative phase; however, data at hand indicate that plasmodia of *S. fusca* remain in the vegetative stage somewhat longer than do plasmodia of *S. axifera*.

Comatricha typhoides (Bull.) Rost.— In a recent paper (Gray, 1937) the writer described the development of this species from white plasmodium to mature sporangia. The time required for completion of fruiting in the specimen described was twenty-three hours. Another such observation has been made, and the required time was fifteen hours. This variation in time was probably due to difference in ages of plasmodia, and since plasmodia of this species are similar in habit to those of various species of *Stemonitis*, it will be difficult to determine the normal length of time which they spend in the vegetative stage.

C. typhoides apparently does not need light in order to fruit, since plasmodia have frequently appeared and eventually formed sporangia on moist pieces of decayed wood kept in the dark.

Lamproderma arcyrionema Rost.— Spores of this species may be germinated easily in either tap- or distilled water, but as yet no plasmodial development has been obtained on any of the artificial media used. Plasmodia have been obtained, however, by sowing spores on small pieces of moist, decayed wood kept in the dark. Sporangia have been obtained in this fashion in the complete absence of light, but no data are available concerning the exact length of the vegetative phase. Like the previously described members of the order Stemonitales, *L. arcyrionema* possesses plasmodia which inhabit the interior of decaying wood; hence the age of plasmodia cannot be accurately determined. Since in several instances small, pearly-white plasmodia of this species arose on moist wood and formed sporangia in 12–14 hours, we may infer that *L. arcyrionema* possesses a relatively short rhythm in so far as the vegetative stage is concerned.

Hemitrichia vesparium (Batsch) Macbr.—The plasmodium of this species is listed in most taxonomic works as being deep red or purple red, but the writer has never observed anything but white plasmodia during the active vegetative phase. It is true that the color becomes red before the fruiting bodies are mature, but this color change occurs after sporangial delimitation has begun and not while plasmodia are still vegetating. It is recognized that plasmodial colors may be somewhat variable, depending upon the color of the substratum, but generally a radical change in plasmodial color during the vegetative phase is due simply to the engulfing of different colored particles of the substratum. Naturally, if a plasmodium should engulf red particles it would, for a time, appear red. This type of change in plasmodial color is exemplified by the photograph of a plasmodium of *F. septica* (fig. 6); at the top the color is brownish-yellow due to the engulfing of many, small, brown fragments of decayed wood on which it was growing before it was allowed to spread to the paper in the moist chamber.

H. vesparium needs no light in order to form sporangia. Numerous instances have occurred in which plasmodia have come to the surfaces of pieces of decayed wood, which had been placed in the dark, and there formed sporangia. Sporangia of *H. vesparium* have also been found on the walls and ceilings of mushroom houses, where the only light was an occasional flashlight beam.

Spore sowings in tubes of corn decoction agar have yielded pearly-white plasmodia which grow rapidly and are easily maintained through plasmodial transplants. In this type of culture fruiting was very erratic, so no data concerning rhythm or effect of light intensity are available. Fruiting frequently occurred, but in no case was an entire plasmodium utilized in sporangial formation. In several cultures, one sporangium was produced by a small bit of plasmodium and then was engulfed and destroyed almost immediately by the remaining plasmodial mass. As

yet the factors inducing fruiting in *H. vesparium* in this type of culture are unknown.

Hemitrichia spp.—Except for the species just discussed, no other species of the genus *Hemitrichia* were grown in the laboratory. However, several naturally occurring plasmodia of *H. clavata* (Pers.) Rost. (fig. 3) and *H. stipitata* (Massee) Macbr. were observed to form sporangia in the complete absence of light. Both species have been found in mushroom houses where they had fruited in darkness. The writer has observed only white plasmodia of either species, although Lister (1925, p. 221) states that the plasmodium of *H. clavata* may be either watery-white or rose-red. Sporangia of both species have been observed to pass through a rose-red stage, but this change of color occurs after sporangial delimitation has started.

Variable plasmodial types. — One species, *Didymium xanthopus* (Ditm.) Fr., has been placed in this separate group because of the variability of its plasmodial color. In the discussions of various yellow-pigmented plasmodial types it was noted that the bright yellow color fades to a certain extent when plasmodia are placed in the light. Plasmodia of *D. xanthopus* did not exhibit this type of variability, as any given plasmodium might vary from white, through various shades of creamy- or greenish-yellow to bright yellow, independently of light conditions. For instance, two plasmodial transplants might be made from a single yellow (or white) plasmodium, and the plasmodium of one of the resultant cultures would be white or cream-colored, whereas the other would be yellow. Plasmodia, regardless of color, formed sporangia equally well under conditions of artificial light or darkness, although there was no regularity in the duration of the vegetative phase of the various cultures—fruiting occurring any time between six and twenty-seven days. Plasmodial cultures were maintained in direct sunlight for periods as long as twenty days, but no cultures ever formed sporangia under such conditions.

Table 5. *Summation of observations on twenty-two plasmodia of T. persimilis which completed their life cycles in complete absence of light.*

No. of plasmodia	Source of plasmodia	Time of collection	Fruiting completed	Hours required
1	Under bark of decaying cottonwood log	2:00 P.M. Sept. 8	8:00 A.M. Sept. 9	18
3	Under bark of decaying wild cherry log	3:00 P.M. Sept. 13	8:00 A.M. Sept. 14	17
1	Under bark of decaying wild cherry log	3:00 P.M. Sept. 13	8:00 A.M. Sept. 15	31
3	Appeared on moist wood in dark chamber	10:00 A.M. Sept. 14	8:00 A.M. Sept. 15	22
7	Under bark of decaying wild cherry log	1:30 P.M. Sept. 15	11:00 P.M. Sept. 15	9.5
3	Under bark of decaying wild cherry log	1:30 P.M. Sept. 15	8:00 A.M. Sept. 16	18.5
4	Under bark of decaying wild cherry log	2:00 P.M. Sept. 17	8:00 A.M. Sept. 18	18

Trichia persimilis Karst.—The plasmodium of this species is typically watery-white and does not change color until the separate sporangia have taken form (fig. 8). After the sporangia have assumed the shape and size of mature fruiting bodies, they become pale lemon-yellow and gradually darken until the shining golden- or yellow-ochraceous color of mature sporangia is attained. Attempts to culture this species in the laboratory on various types of media used for other species have failed, but numerous observations have been made on plasmodia collected in the field and brought to the laboratory. These plasmodia were all collected beneath the bark of decaying logs, where they received no light; they were then transported in darkness to the laboratory where most of them were placed in moist chambers in the dark. Fruiting bodies were formed both by sporangia kept in the dark and those kept in diffuse natural light. Since the age of each plasmodium is unknown, no definite rhythm may be set for *T. persimilis;* the indications are, however, that it has a comparatively short rhythm. Table 5 is a summation of the observations made on twenty-two plasmodia of this species which fruited in the dark.

It is evident from these results that light plays no important role in sporangia formation, and at present no theory is presented as an explanation of what causes fruiting in this species. There is a possibility that temperature is an important factor, since Skupienski (1934) maintains that different strains of this species have different optimum temperatures, although the race which he terms "*thermophilum*" (not implied in a systematic sense) showed very little variation in length of vegetative phase under temperature conditions varying from 6° to 25°C. If physiologic strains do exist, the interpretation of the results of other investigations, as well as the present

one, becomes a difficult task, since different strains may be involved in the various studies.

Discussion. — From the above observations, it is evident that under the conditions outlined yellow-pigmented plasmodia will not form fruiting bodies in the complete absence of light. This leads immediately to the conclusion that the pigment has an important function and is probably a part of the mechanism by which plasmodia are able to perceive the stimulus of light. The fundamental role of pigments in various living processes has been shown with many organisms, plant and animal, so this finding is not that of a new phenomenon but merely another example of a long-known one.

The chemical nature of the pigment has not been determined, but several theories have been advanced as to the nature of pigments that occur in myxomycetes. Seifriz and Zetzmann (1935) suggested that the yellow pigment of *Physarum polycephalum* belongs to the lyochrome or flavone group (of which the flavins are members). If the pigment is of the flavone type, a plant relationship would be suggested for the group, since such pigments as a rule do not occur in animal tissues. Solacolu (1932), working with pigments from the mature fruiting bodies of twenty-six species of myxomycetes, came to the conclusion that pigments occurring in these organisms are anthracenes. He also suggested a plant relationship, since anthracenes are found in some fungi and some higher plants, but generally not in animals, although phenanthrene, an isomer of anthracene, and its derivatives are known to occur in animals (Fieser, 1936). Whatever the chemical nature of these pigments may be, it is evident that light has a specific photochemical effect upon them, since plasmodia were observed to become paler when exposed to light. Stobbe (1908), working with various fulgides, has shown that these complex chemical substances not only change color when exposed to light but also that lights of various wave lengths cause different changes. For example, this worker found that triphenylfulgid became orange-yellow when exposed to light of 550 to 700 millimicrons but became dark brown at wave lengths of 440 to 550 millimicrons. The fact, as shown by Stobbe, that light of various wave lengths may induce specific photochemical effects may explain in part the behavior of plasmodia of *P. polycephalum* when exposed to the different wave bands. The work of Brandza (1926) would indicate that profound morphological changes may occur in myxomycetes as a result of the influence of light. This worker reports that plasmodia of some species can live in direct sunlight and that such plasmodia are extremely modified. For example, he states that *Physarum viride* (Bull.) Pers. and its variety *incanum* Lister are really forms of *P. nutans* Pers., which were derived from different colored plasmodia by exposure to direct sunlight. Such change in color indicates a definite photochemical effect.

It was noted that yellow-pigmented forms showed a tendency to clump when exposed to light, a finding in agreement with the results reported by other investigators. Baranetzki (1876), working with *Fuligo septica*, first mentioned clumping of protoplasm of plasmodia; this worker further stated that a slight increase in illumination causes a distinct retardation of streaming movements of plasmodia. Englemann (1879), working with the amoeba, *Pelomyxa palustris*, noted that when light is thrown upon a pseudopod of this organism, it is withdrawn suddenly. Mast (1911, p. 229) makes the general statment that light retards the activity of protoplasm and prevents the formation of pseudopods on the more highly illuminated side. Thus we can see that light causes a contraction of protoplasm of organisms other than myxomycetes, and, while it may simply be an orientation process with respect to the stimulus of light, it would appear to be more than a coincidence that plasmodia not only clump when exposed to light but also when about to form fruiting bodies, as Baranetzki has already pointed out.

Various workers (Baranetzki, 1876; Stahl, 1884, p. 167; Davenport, 1879) have shown that plasmodia of myxomycetes, which are known to react to light, are negatively phototropic. The anomaly presented by a negatively phototropic organism requiring light for one of its most important processes is evident. The fact that the above workers have demonstrated this negative phototropism does not necessarily mean that plasmodia will always react negatively, however, since it is known that certain organisms may change their tropic responses. For example, Keeble (1910, p. 41-42), working with two species of *Convoluta*, found that in bright light *C. roscoffensis* is positively and *C. paradoxa* negatively phototropic, while in dim light, *C. roscoffensis* is non-phototropic and *C. paradoxa* is positively phototropic. Loeb (1904) has shown that certain fresh-water crustaceans (*Gammarus*, *Cyclops*, *Daphnia*), which are naturally negatively phototropic, can be induced to reverse their tropic responses through the use of certain chemicals, particularly acids. It may well be that myxomycetes, like other organisms, have the power of changing their phototropic responses.

The fact that under controlled conditions of light, temperature, and nutrition, plasmodia fruited with great regularity would support the earlier view of Cayley (1929) that a certain periodicity (rhythm) exists in the production of spores. Among the Fucaceae, the periodical liberation of sexual cells has been described by several workers. Williams (1898, 1905), who noted a fortnightly liberation of sexual cells of *Dictyota dichotoma* off the coast of England and Wales, concluded that this periodicity was hereditary, but that in seas where there are tides, it might be influenced by the changes of amount of illumination. Hoyt (1907) described a monthly liberation of sexual cells of this same species at Beaufort, North Carolina, and Lewis (1910) found that it produced fortnightly crops at Naples and noted that the critical points in the life cycle (initiation of sexual cell rudiments and liberation) not only bore a relationship to the tides,

as shown by previous workers, but coincided exactly with the periods of maximum intensity of illumination. This latter worker hypothecated that *Dictyota*, in adapting itself to differing conditions at various localities, has acquired its habit of periodicity in response to different factors. Tahara (1909) described an identical periodicity in the fortnightly liberation of oogonia of *Sargassum enerve;* in a later work (1913), Tahara showed that the initial liberation in *S. enerve, S. Horneri,* and *Cystophyllum sisymbrioides* bore a relationship to the highest spring tide, but that the intervals between successive liberations varied, thus suggesting the possible influence of other environmental factors (illumination?). Keeble (1910, p. 33) found that light was a very important factor in the egg-laying of *Convoluta roscoffensis*. He found that alternation of six hours of exposure to light with eighteen hours of darkness was the optimum for egg-laying and noted that this was the same exposure received by *C. roscoffensis* during spring tidal periods at which its eggs are laid habitually.

In relation to the rhythmical fruiting of myxomycetes, Williams' view that periodicity might be hereditary is supported in part by the fact that certain species exhibit longer vegetative phases than others. For example, *Physarum tenerum*, under a 100-watt light, required eighteen days for fruiting, whereas, *P. polycephalum*, under identical conditions, required only eleven days. While rhythmical fruiting may possibly be hereditary to a certain extent, it is certainly not so innate as to be unalterable with respect to environmental factors. The writer has shown that in fed cultures of *P. polycephalum* fruiting rhythm may be changed by varying the intensity of illumination. Camp (1937) showed that by starvation of plasmodia the vegetative phase of this species could be markedly reduced. Experiments of the writer, involving both starvation and intensity of illumination, showed that both factors inter-reacted in influencing the time of fruiting. Differences in rhythms of species, then, tend to support the view that rhythmical fruiting is hereditary; yet experiments on yellow-pigmented plasmodia, involving lights of different intensities, show that this periodicity of fruiting may be modified.

SUMMARY

Our lack of knowledge of the biology of the myxomycetes is due to the fact that so few species have been cultured. The problem of immediate interest is the development of methods for culturing many species under laboratory conditions.

The effects of light upon the fruiting rhythms of four yellow-plasmodial types, ten non-pigmented plasmodial types, and one variable plasmodial type (*Didymium xanthopus*) were investigated. It was found that, with the species studied, yellow-pigmented types require light in order to complete their life cycles, while non-pigmented types and *D. xanthopus* fruit equally well in light or darkness.

Under controlled conditions of temperature, light, and nutrient, fruiting periods of pigmented types assumed great regularity. Under conditions of constant temperature and continuous illumination the lengths of the vegetative phase of *Physarum polycephalum* and *P. tenerum* are conditioned by the total amount of light received. Under conditions of intermittent illumination the vegetative phase of *P. polycephalum* may be lengthened, although the total amount of light necessary for a cycle to be completed may be greatly reduced. Fed cultures of *P. polycephalum*, when placed in lights of various wave lengths, formed sporangia only when exposed to the shorter wave lengths of the visible spectrum. The plasmodia of all yellow-pigmented types clumped and faded when they were exposed to light.

The laboratory cultivation of *Physarum compressum*, *P. tenerum*, and *Hemitrichia vesparium* is reported for the first time.

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LITERATURE CITED

Baranetzki, J. 1876. Influence de la lumière sur les plasmodia des Myxomycètes. Mem. Soc. Nat. Sc. Natur. Cherbourg 19: 321–360.

Brandza, M. 1926. Sur la polychromie des myxomycètes vivant en plein seleil. Compt. Rend. Acad. Sci. Paris 182: 987–989.

Camp, W. G. 1936. A method of cultivating myxomycete plasmodia. Bull. Torrey. Bot. Club 63: 205–210.

———. 1937. The fruiting of *Physarum polycephalum* in relation to nutrition. Amer. Jour. Bot. 24: 300–303.

Cayley, Dorothy M. 1929. Some observations on mycetozoa of the genus *Didymium*. Trans. British Mycol. Soc. 14: 227–248.

Davenport, C. B. 1897. Experimental morphology. Vol. I. New York.

Englemann, T. W. 1879. Über Reizung contraktilen Protoplasmus durch plotzliche Beleuchtung. Arch. Ges. Physiol. 19: 1–7.

Fieser, L. F. 1936. The chemistry of natural products related to phenanthrene. New York.

Gray, W. D. 1936. Notes on plasmodial behavior of *Stemonitis fusca* Roth. Proc. Indiana Acad. Sci. 45: 74–76.

———. 1937. Observations on the methods of stipe-formation in *Stemonitis* and *Comatricha*. Proc. Indiana Acad. Sci. 46: 81–85.

Hofmeister, W. F. B. 1867. Lehre von der Pflanzenzelle. Leipzig.

Howard, F. L. 1931. Laboratory cultivation of myxomycete plasmodia. Amer. Jour. Bot. 18: 624–628.

Hoyt, W. D. 1907. Periodicity in the production of the sexual cells of *Dictyota dichotoma*. Bot. Gaz. 43: 383–392.

Keeble, F. 1910. Plant animals. A study in symbiosis. University Press. Cambridge.

Klebs, G. 1900. Zur Physiologie der Fortpflanzen einiger Pilze. III. Allg. Betrachtungen Pringsh. Jahrb. 35: 80–203.

Lewis, I. F. 1910. Periodicity in *Dictyota* at Naples. Bot. Gaz. 50: 59–64.

Lister, A. 1888. Notes on the plasmodium of *Badhamia* and *Brefeldia*. Annals Bot. 2: 1–24.

———. 1925. Monograph of the mycetozoa. 3rd. edition, revised by G. Lister. Oxford University Press.

Loeb, J. 1904. The control of heliotropic reactions in fresh water crustaceans by chemicals, especially CO_2. Univ. California Publ. Physiol. 2(1): 1–3.

Mast, S. O. 1911. Light and the behavior of organisms. New York.

Seifriz, W., and M. Russell. 1936. The fruiting of myxomycetes. New Phytol. 35: 472–478.

———, and M. Zetzmann. 1935. A slime-mould pigment as indicator of acidity. Protoplasma 23: 175–179.

Skupienski, F. X. 1920. Recherches sur le cycle évolutif de certains myxomycètes. Paris.

———. 1928. Badania Bio-cytolicze nad *Didymium difforme*. Cześć pierwsza. (Étude bio-cytologique du *Didymium difforme*. Première partie.) Acta. Soc. Bot. Poloniae 5: 255–336.

———. 1934. Sur l'existence de races physiologiques chez les Myxomycètes. Annales de Protistologie 4. 121–132.

Solacolu, T. 1932. Sur les matières colorantes de quelques myxomycètes. Le Botaniste 24: 107–140.

Stahl, E. 1884. Zur Biologie der Myxomyceten. Zeitschr. Bot. 42: 146–155, 162–175, 187–191.

Stobbe, H. 1908. Phototropieerscheinungen bei Fulgiden und anderen Stoffen. Liebig's Annalen der Chemie, Bd. 359: 1–48.

Tahara, M. 1909. On the periodical liberation of the oospheres in *Sargassum*. Bot. Mag. Tokyo 23: 151–153.

———. 1913. Oogonium liberation and the embryogeny of some fucaceous algae. Jour. Coll. Sci. Univ. Tokyo 32 (9): 1–13.

Williams, J. L. 1898. Reproduction in *Dictyota dichotoma*. Annals Bot. 12: 559–560.

———. 1905. Studies in the Dictyotaceae. III. The periodicity of the sexual cells of *Dictyota dichotoma*. Annals Bot. 19: 531–560.